

What is claimed is:

1. A DNA fragment comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID No. 1 or a homologue thereof.
2. A DNA fragment according to Claim 1 comprising the nucleotide sequence of SEQ ID No. 2.
3. A DNA encoding the amino acid sequence of SEQ ID No. 1 or a homologue thereof.
4. A DNA according to Claim 3 comprising the nucleotide sequence of SEQ ID No. 2.
5. A polypeptide comprising the amino acid sequence of SEQ ID No. 1 or a homologue thereof.
6. A recombinant plasmid wherein a DNA according to Claim 3 or 4 is integrated into the plasmid vector.
7. A host cell transformed by a recombinant plasmid according to Claim 6.
8. A process for producing a β -fructofuranosidase comprising:
cultivating a host cell according to Claim 7, and
collecting the β -fructofuranosidase from the host and/or the culture thereof.
9. A process for producing fructooligosaccharides comprising a step of bringing into contact with sucrose a host cell according to Claim 7 or β -fructofuranosidase obtained in Claim 8.
10. A process for isolating a β -fructofuranosidase gene by making use of the homology thereof to a nucleotide sequence comprising all or part of the nucleotide sequence of SEQ No. 2.
11. A process according to Claim 10 comprising:

preparing a gene library which presumably contains a β -fructofuranosidase gene,

screening the gene library using a nucleotide sequence comprising all or part of the nucleotide sequence of SEQ ID No. 2 to select sequences which hybridize with the nucleotide sequence comprising all or part of the nucleotide sequence of SEQ ID No. 2 from the gene library, then isolating the selected sequences, and

isolating a β -fructofuranosidase gene from the sequences which have been selected and isolated from the gene library.

12. A process according to Claim 11 wherein the gene library is a genomic DNA library or a cDNA library.

13. A process according to Claim 10 comprising:

preparing a primer consisting of a nucleotide sequence which comprises all or part of the nucleotide sequence of SEQ ID No. 2,

carrying out PCR process on the primer using a sample which presumably contains a β -fructofuranosidase gene as a template, and

isolating a β -fructofuranosidase gene from the amplified PCR product.

14. A process according to any one of Claims 11 to 13 wherein the gene library which presumably contains a β -fructofuranosidase gene or the sample which presumably contains a β -fructofuranosidase is derived from a Eumycetes species.

15. A process according to Claim 14 wherein the Eumycetes species is an Aspergillus, Penicillium or Scopulariopsis species.

16. A polypeptide comprising the amino acid sequence of SEQ ID No. 11 or a homologue thereof.

17. A DNA encoding a polypeptide according to Claim 16.

18. A DNA according to Claim 17 comprising the nucleotide sequence of SEQ ID No. 12.

19. A polypeptide comprising the amino acid sequence of SEQ ID No. 13 or a homologue thereof.

20. A DNA encoding a polypeptide according to Claim 19.

21. A DNA according to Claim 20 comprising the nucleotide sequence of SEQ ID No. 14.

22. An *Aspergillus* mold fungus without β -fructofuranosidase activity.

23. A mold fungus according to Claim 22 which has been deprived of β -fructofuranosidase activity by deleting all or part of the β -fructofuranosidase gene on the chromosome DNA of the original *Aspergillus* mold fungus.

24. A mold fungus according to Claim 23 which is *Aspergillus niger*.

25. A mold fungus according to Claim 24 which is *Aspergillus niger* NIA1602 (FERM BP-5853).

26. A process for producing a β -fructofuranosidase comprising:

transforming a mold fungus according to any one of Claims 22 to 25 using a DNA construction comprising a DNA encoding a β -fructofuranosidase,

cultivating the transformant, and

collecting the β -fructofuranosidase from the transformant and/or the culture thereof.

27. A β -fructofuranosidase variant having fructosyltransferase activity obtained by a mutation in the original β -fructofuranosidase thereof,

wherein the mutation comprises an insertion, substitution or deletion of one or more amino acids in, or an addition to either or both of the terminals of, the amino acid sequence of the original

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β -fructofuranosidase, and

the variant makes the composition of the fructooligosaccharide mixture produced from sucrose as a result of fructosyltransfer reaction by the variant β -fructofuranosidase different from the composition of the fructooligosaccharide mixture produced by the original β -fructofuranosidase.

28. A β -fructofuranosidase variant according to Claim 27 which improves the selectivity and/or efficiency of 1-kestose in the fructooligosaccharide mixture.

29. A β -fructofuranosidase variant according to Claim 27 or 28 wherein the original β -fructofuranosidase is derived from a Eumycetes species.

30. A β -fructofuranosidase variant according to Claim 29 wherein the original β -fructofuranosidase is derived from an Aspergillus, Penicillium, Scopulariopsis, Aureobasidium or Fusarium species.

31. A β -fructofuranosidase variant according to Claim 30 wherein the original β -fructofuranosidase is the β -fructofuranosidase consisting of the amino acid sequence of SEQ ID No. 1 or a homologue thereof.

32. A β -fructofuranosidase variant according to Claim 31, wherein one or more amino acid residues at the positions selected from the group consisting of positions 170, 300, 313 and 386 in the amino acid sequence of SEQ ID No. 1, or, for a homologue of the amino acid sequence of SEQ ID No. 1, or one or more amino acid residues at the positions selected from the group consisting of the positions equivalent to the positions 170, 300, 313 and 386, are substituted by other amino acids.

33. A β -fructofuranosidase variant according to Claim 32, wherein amino acid residue at position 170 in the amino acid sequence of SEQ ID No. 1 or the amino acid residue at the position

equivalent to position 170 is substituted by an aromatic amino acid selected from the group consisting of tryptophan, phenylalanine and tyrosine.

34. A β -fructofuranosidase variant according to Claim 32, wherein amino acid residue at position 300 in the amino acid sequence of SEQ ID No. 1 or the amino acid residue at the position equivalent to position 300 is substituted by an amino acid selected from the group consisting of tryptophan, valine, glutamic acid and aspartic acid.

35. A β -fructofuranosidase variant according to Claim 32, wherein amino acid residue at position 313 in the amino acid sequence of SEQ ID No. 1 or the amino acid residue at the position equivalent to position 313 is substituted by a basic amino acid selected from the group consisting of lysine, arginine and histidine.

36. A β -fructofuranosidase variant according to Claim 32, wherein amino acid residue at position 386 in the amino acid sequence of SEQ ID No. 1 or the amino acid residue at the position equivalent to position 386 is substituted by a basic amino acid selected from the group consisting of lysine, arginine and histidine.

37. A β -fructofuranosidase variant according to Claim 32, wherein amino acid residues at positions 170, 300 and 313 in the amino acid sequence of SEQ ID No. 1 or the amino acid residues at the positions equivalent to positions 170, 300 and 313 are substituted by tryptophan, tryptophan and lysine, respectively.

38. A β -fructofuranosidase variant according to Claim 32, wherein amino acid residues at the positions 170, 300 and 313 in the amino acid sequence of SEQ ID No. 1 or the amino acid residues at the positions equivalent to positions 170, 300 and 313 are substituted by tryptophan, valine and lysine, respectively.

39. A DNA encoding a variant β -fructofuranosidase according to any one of Claims 27 to 38.

40. A vector expressing a variant β -fructofuranosidase which comprises a DNA according to Claim 39.

41. A host cell comprising an expression vector according to Claim 40.

42. A host cell according to Claim 41 wherein the host cell is a mold fungus according to any one of Claims 22 to 25.

43. A process for producing a variant β -fructofuranosidase according to any one of Claims 27 to 38 comprising:

transforming a host cell using a DNA according to Claim 39 or an expressing vector according to Claim 40,

cultivating the transformant, and

collecting the β -fructofuranosidase from the transformant and/or the culture thereof.

44. A process for producing a variant β -fructofuranosidase according to Claim 43 wherein the host cell is a mold fungus according to any one of Claims 22 to 25.

45. A process for producing fructooligosaccharides comprising bringing into contact with sucrose a host cell according to Claim 41 or 42 or a variant β -fructofuranosidase according to any one of Claims 27 to 38.

46. A mold fungus according to any one of Claims 22 to 25 transformed by a DNA fragment or a DNA according to any one of Claims 1 to 4.

47. A process for producing a β -fructofuranosidase comprising:

cultivating a mold fungus according to Claim 46, and

collecting the β -fructofuranosidase from the mold fungus and/or the culture thereof.

49. A process for producing a β -fructofuranosidase comprising:

comprising:
cultivating a mold fungus according to Claim 48, and
collecting the β -fructofuranosidase from the mold fungus
and/or the culture thereof.

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